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(54) Title: USE OF NGF FOR THE MANUFACTURING OF A DRUG FOR TREATING ALLERGIC DISORDERS

(57) Abstract: The invention relates to the use of nerve growth factor for the manufacturing of a drug for treating allergic disorders in human beings and animals. Preferably, the drug is administrated to the site of action at a nerve growth factor concentration which does not result in the perception of pain.

USE OF NGF FOR THE MANUFACTURING OF A DRUG FOR TREATING ALLERGIC DISORDERS

The invention relates to a new use of nerve growth factor. More specifically, the invention relates to the use of nerve growth factor for the manufacturing of a drug for treating allergic disorders in human beings and animals. Preferably, the drug is administrated to the site of action at a nerve growth factor concentration which does not result in the perception of pain.

Nerve growth factor is one of the growing family of neurotrophins. It was discovered by professor Rita Levi-Montalcini in salivary glands. Previous studies have focused on its role in neurogenesis and nerve differentiation. It has been demonstrated to actively participate in the regulation of acetylcholine containing nerves in the brain of adults. In the periphery, the function of nerve growth factor has been associated with proinflammatory and pain effects.

Allergic disorders represent a major health problem and have a high social and economical cost. Thus, there is a demand for remedies to prevent, overcome or reduce these types of disorders, which would be of great clinical interest. For example, immune corneal ulcers are rare ocular surface diseases with multiple etiologies which are accompanied by diverse pathogenesis. Immunosuppressive drugs and systemic or topical steroids may occasionally control the inflammatory process, but in the more severe cases the ulcer may progress to corneal melting and perforation. No suitable therapy is available at present for these patients.

Allergic disorders are either genetically determined or provoked by chemical compounds, physical or surgical injuries as well as by the administration of currently used chemotherapeutic compounds. These allergic disorders are very often initially associated with local inflammation,

accumulation of immunocompetent cells and overexpression of endogenous molecules locally released by these or other cells and later by degeneration.

Available evidence obtained in a variety of animal models and human pathologies indicate that nerve growth factor (NGF) is constantly expressed after nerve injury and during inflammatory responses. However, while the crucial role of NGF in growth and differentiation of peripheral nerves has been well established, the role of NGF during the inflammatory status is still a matter of debate. Evidence for a harmful as well as a helpful effect has been reported. Also, the role of NGF following the inflammatory status, i.e. in the degenerative and reparatory phase, is not known.

It is known that NGF triggers acute pain by changing sensitivity in the nociceptor. NGF has also been shown to play a role in the plasticity of the central nervous system in chronic pain. However, there is now also increasing evidence for profound changes of the primary sensory neurons including nociceptors throughout the life of an organism, and these changes account for clinically relevant alterations of pain perception.

In adults NGF acts as an important intermediate in inflammatory pain, contributing to both peripheral and central sensitization. The sensitization of peripheral nociceptors can be very rapid and can involve non-neural cells such as mast cells, neutrophils, fibroblasts, and macrophages. Recent evidence indicates that other neurotrophins also play key supporting roles in the plasticity of nociceptors and in inflammatory pain.

There is growing evidence that NGF may function as a mediator of persistent pain states and NGF-antagonists alleviate pain. The expression of NGF also correlates with pain in human pancreatic cancer.

In a recent uncontrolled study (N. Eng. J. Med. (1998) 338:1174) topically applied exogenous NGF restored corneal innervation of patients with corneal neurotrophic ulcers. Nerve growth factor might promote corneal healing and is implicated in functional activity of inflammatory cells on the ocular surface (Invest. Ophthalmol. Vis. Sci. (1998) 39:1272). It was demonstrated that NGF induced recovery on sensory nerve impairment in the cornea of patients affected by neurotrophic keratitis. In this disease the impairment of ophathalmolic branch of the trigeminal nerve is known to play a key role in inducing the pathological changes at the corneal epithelium and stroma.

Inflammatory responses to injury are known to be beneficial to axonal regeneration. In peripheral nerve injury macrophages migrate into the distal segment of the interrupted nerve, participate in the removal of axons debris, stimulate the proliferation of Schwann's cells, and augment the synthesis of NGF by non-neuronal cells. Macrophages, which appear in injured regions of the peripheral and central nervous system after axotomy, are though to play a crucial role in the process of neural regeneration *in vivo* by secreting a variety of biologically active molecules.

Nerve growth factor is produced and released by for example macrophages and mast cells. Biological compounds released by these latter cells, including most probably NGF, are potent stimulatory agents for fibroblast proliferation. The NGF released by macrophages participates in the nerve repair of rat intestines infected with parasites.

Nerve growth factor has been shown to inhibit some acute experimental inflammation, suggesting that NGF play a positive functional role by reducing vascular permeability during acute inflammation. Nerve growth factor is produced by mast cells during cutaneous inflammation. Under these conditions of neuronal damage and repair the long term NGF

release by mast cells seems to provide a mechanism by which the local and harmful effect of the proinflammatory toxic effect of cytokines might be limited. An anti-inflammatory role of NGF has been suggested (Arch. Int. Pharmacodynam. Ther. (1989) 299:269), also by recent observations demonstrating that NGF can inhibit mast cell TNF-α production even in the context of degranulation stimulus of high doses of substance P (Rheumatol. Int. (1995) 14:249).

In WO 98/46254 a process is shown for preventing in humans the demyelination of nerve fibres with NGF or active NGF fragments. This is accomplished by pharmaceutical compositions of NGF, which influence the immune system and enter the blood brain barrier.

Nerve growth factor or NGF fragments have also been shown to stimulate osteogenesis. JP 7242564 depicts the ability of NGF to stimulate alkaline phosphatase and accelerate collagen synthesis.

The purpose of the present invention is to provide remedies for allergic disorders. Furthermore, the invention provide remedies for such disorders without evoking pain.

When a nerve growth factor is used according to the invention in a drug for treating allergic disorders the drug can be supplied to the site of action systemically as well as locally. For example, topical application of NGF promotes the corneal healing and recovery in ocular allergic diseases unresponsive to existing therapies.

Nerve growth factor is implicated in the regulation of reactions leading to the anti-allergic activity and the start of the curing of allergic disorders.

According to the invention a nerve growth factor can be used in a drug as a bioactive component which participates in the healing of allergic disorders. This surprising and crucial role of NGF in reparative mechanisms is

supported by the observation that corneal epithelial cells are receptive to the action of NGF.

Nerve growth factors works within the physiological range since the dose necessary to produce its effect is similar to the amount of NGF-1 present in the blood circulation of adult animals. When a nerve growth factor is used for the manufacturing of a drug for treating allergic disorders according to the invention the drug is administrated to the site of action at a NGF concentration of up to 1,000 μg/ml, preferably up to 500 μg/ml, most preferred between 10 and 250 μg/ml.

However, the nerve growth factor is preferably supplied to the site of action at a concentration which does not activate and/or stimulate human nociceptors. When the drug comprising nerve growth factor is administrated to the site of action at a NGF concentration which does not result in the perception of pain the NGF concentration is less than 100 μg/ml.

The allergic disorder can be a local and systemic allergic disease, for example a corneal ulcer.

Examples of disorders to be treated according to the invention are osteoarthrosis, osteoporosis, defect fracture healing, venous ulcer, diabetic ulcer, and decubitus pressure ulcer.

The allergic disorder can also be conjunctivitis, dermatitis and hypersensitivy to drugs. Other disorders which can be prevented, overcome or reduced according to the invention are allergic inflammatory arthritis, such as rheumatoid arthritis, juvenile arthritis, systemic lupus erythematosus, systemic sclerosis, polymyositis, necrotitizing vasculitis, Chron's disease, ulcerous colitis, irritable bowel syndrome, allergic changes in the gastrointestinal tract, degenerative bladder, degenerative changes in the bladder, Sjogren's disease, psoriasis, and sarcoidosis.

The allergic disorder can also be a degenerative lesion in the skin.

Nerve growth factor also contributes to the repair of the nervous system since NGF is produced, released and used by immunocompetent cells and its levels undergoes variation. Nerve growth factor released by inflammatory cells plays a role in these reparative events since an inflammation near the nerve cell body enhances axonal regeneration.

Nerve growth factor exerts this beneficial effect including its anti-allergic activity on local "brain inflammatory responses" such as Alzheimer's disease.

The effect obtained by NGF on human ocular degeneration is due to a mechanism involving an anti-allergic effect. Here, the corneal ulcer is the result of an allergic reaction at the ocular surface which causes the release of toxic cell mediators. In this case the involvement at sensory nerves level is only secondary to the corneal damage and the healing effect of NGF is associated with the anti-allergic and reparative properties of NGF as well as with its effect on restoring corneal epithelial cell damages and/or cell replacement.

Nerve growth factor induces proliferation and differentiation of corneal epithelial cells. The topical application of NGF blocks the inflammatory condition and promotes healing within 2 weeks in patients affected by severe corneal ulcers with stromal melting caused by multiple immune etiologies, and unresponsive to steroid and immunosuppressive drugs. A good clinical response is obtained in patients treated with nerve growth factor.

For the above-described uses nerve growth factors are provided as pharmaceutical preparations. A pharmaceutical preparation of NGF may be administrated alone or in a combination with pharmaceutically acceptable carriers, in either single or multiple doses. Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile

aqueous solution and balanced salt solutions, such as PBS, PBSS, GBSS, EBSS, HBSS, or SBF. The pharmaceutical compositions formed by combining a nerve growth factor and the pharmaceutically acceptable carriers are then easily administrated in a variety of dosage forms. The administration may be intravenous, intraperitoneal, parental, intramuscular, subcutaneous, oral, or topical. Topical and intravenous administrations are preferred.

EXAMPLES

The following non-limiting examples will now be given in order to further describe the invention.

Example 1. Effect of NGF on rat paw oedema.

Nerve growth factor is equally effective given locally or systematically in an animal model of rat paw oedema. The powerful anti-inflammatory activity of NGF is 1000 times more effective than indomethacin and 10 times more effective that dexamethasone. It is considerable more active than the non-steroidal drugs in clinical use and is more active also than substances that are counter irritant in animal models of inflammation.

When administered systemically at the same time as the irritant the anti-inflammatory activity of NGF is not seen at doses over 25 μg/kg. This finding entails that the dose response curve of this particular route of administration is bell-shaped.

Example 2. NGF as an active inhibitor of mitogen-activated protein kinase p38.

Tumor necrosis factor-alpha (TNF-alpha) is a cytokine secreted by activated monocytes/macrophages and T lymphocytes. It has been implicated in several disease states, including rheumatoid arthritis, inflammatory bowel disease, septic shock, and osteoporosis. A monocyte/macrophage production of TNF-alpha is dependent on the mitogen-activated protein kinase p38.

Nerve growth factor at a concentration of 250 µg/ml inhibited the release of TNF-alpha by lipopolysaccharide (a monocyte stimulus) treated human peripheral blood mononuclear cells with an IC(50) value of 4 nM. Likewise, NGF inhibited the release of TNF-alpha from peripheral blood mononuclear cells, which had been treated with the superantigen staphylococcal enterotoxin B (a T cell stimulus), with an IC(50) value of 15 nM. In all p38 dependent *in vitro* systems tested NGF was approximately 10-fold more potent than the standard p38 kinase inhibitor SB 203580 according to the state of the art. NGF inhibited the enzymatic activity *in vitro* of recombinant p38alpha and beta, but not gamma or delta, and had no significant activity against a variety of other enzymes. NGF did not inhibit T cell production of interleukin-2 or interferon-gamma and did not inhibit T cell proliferation in response to mitogens. However, in mice and rats injected with lipopolysaccharide NGF inhibited the TNF-alpha production. In mice the inhibition was 73% at a NGF concentration of 250 µg/ml, and in rats the inhibition was 78% at a concentration of 125 µg/ml.

These favorable biological anti-osteoporotic properties (oestrogen substitution) clearly demonstrates the use of NGF as a treatment for allergic diseases.

Example 3. Effects of NGF treatment on bone mass and bone mechanical and histomorphometric indices.

The effects of NGF treatment on bone mass and bone mechanical and histomorphometric indices in mature ovariectomized rats with established osteopenia on a low-calcium diet were investigated during 16 weeks.

The therapeutic effects of NGF at a concentration of 50 µg/ml on bone mass and strength, bone metabolic markers, and indices of histomorphometry were investigated in ovariectomized (ovx) rats on a low calcium (0.1%) diet in com-

parison with 17alpha-ethynylestradiol (EE) or 1alpha-hydroxyvitamin D3 [1alpha(OH)D3]. NGF (1 000 µg/kg/day), EE (0.1 mg/kg/day), or 1alpha(OH)D3 (0.5 µg/kg/day) was administered orally once a day for 16 weeks, starting 12 weeks after ovariectomy when the bone mineral density (BMD) of lumbar vertebrae (L4-5) and femur (global, proximal, and distal regions) had already been decreased by the combination of ovariectomy and low dietary calcium.

The BMD of the lumbar vertebrae and the femur was higher in the groups treated with NGF, EE, or 1alpha(OH)D3 than in the ovx control group. The BMD of the mid-diaphysial regions of femur and tibia, which mainly consist of cortical bone, was decreased 28 weeks after ovariectomy in the ovx control group.

The BMD of the mid-diaphysial femur was higher in the groups treated with 1alpha(OH)D3, and the BMD of mid-diaphysial tibia was higher in the groups treated with NGF or 1alpha(OH)D3 than in the ovx control group. As with BMD, the compressive strength of the vertebral body of L2, corrected for the volume of each individual vertebra tested, was higher in the groups treated with NGF, EE, or 1alpha(OH)D3 than in the ovx control group. The trabecular bone volume and the trabecular number were reduced 12 and 28 weeks after ovariectomy, but there was no change in trabecular thickness. These reduced indices were increased in the groups treated with NGF, EE, or 1alpha(OH)D3 when compared with the ovx control group. NGF or EE decreased the serum levels of osteocalcin and bone alkaline phosphatase and the urinary levels of deoxypyridinoline and pyridinoline in comparison with the ovx control group. Furthermore, NGF or EE decreased the mineralizing surface and the bone formation rate as well as the osteoclast surface and osteoclast numbers. However, 1alpha(OH)D3, did not affect these serum and urinary parameters.

These data suggest that NGF suppresses the accelerated bone turnover which is induced by a combination of ovariectomy and low dietary calcium. Furthermore, they indicate that NGF may be a potentially useful drug for the treatment of postmenopausal osteoporosis.

Example 4. Protective effect of NGF on multiple organ failure after zymosan-induced peritonitis in the rat.

A study was performed on the role of NGF in the pathogenesis of multiple organ failure which was induced by peritoneal injection of zymosan in rats.

Animals were randomly divided into six groups (ten rats in each group). The first group was treated with an intraperitoneal (ip) administration of a saline solution (0.9% NaCl) and served as a sham group. The second group was treated with an ip administration of zymosan (500 mg/kg suspended in a saline solution). In the third and fourth groups, the rats received an ip administration of NGF 1 and 6 hrs after the zymosan or saline administration, respectively. In the fifth and sixth groups, the rats received an ip administration of nicotinamide (50 mg/kg) 1 and 6 hrs after the zymosan or saline administration, respectively. After the injection of zymosan or saline the animals were monitored for 72 hrs to evaluate systemic toxicity (conjunctivitis, ruffled fur, diarrhea, and lethargy), loss of body weight, and mortality.

The zymosan administration induced a severe inflammatory response, which was characterized by peritoneal exudation, high plasma and peritoneal levels of nitrate/nitrite (the breakdown products of nitric oxide), and leukocyte infiltration into peritoneal exudate. This inflammatory process coincided with the damage of lung, small intestine, and liver as assessed by a histologic examination and by an increase of myeloperoxidase activity, which is indicative of neutrophil infiltration. Rats treated with zymosan

showed signs of systemic illness, significant loss of body weight, and high mortality rates.

The peritoneal administration of zymosan in rats also induced a significant increase in the plasma levels of peroxynitrite as measured by the oxidation of the fluorescent dihydrorhodamine 123. An immunohistochemical examination demonstrated a marked increase in the immunoreactivity to nitrotyrosine, a specific "footprint" of peroxynitrite, in the lungs of rats shocked with zymosan.

An *in vivo* treatment with NGF (1 and 6 hrs after zymosan injection) or nicotinamide (50 mg/kg, 1 and 6 hrs after the zymosan injection) significantly decreased the mortality, inhibited the development of peritonitis, and reduced the peroxynitrite formation. In addition, PARS inhibitors were effective in preventing the development of organ failure caused by tissue injury and neutrophil infiltration, as evaluated by assaying myeloperoxidase, were reduced in the lung, the small intestine, and the liver.

In conclusion, the NGF activation exerts a role in the development of multiple organ failure and the PARS inhibition is an effective anti-inflammatory anti-oedema therapeutic tool.

Example 5. Enhancement of the contact hypersensitivity reaction by acute morphine administration and inhibition by NGF at the elicitation phase.

The effects of morphine on the irritant contact sensitivity (ICS) and contact hypersensitivity (CHS) reaction was investigated. ICS was induced by the application of croton oil on the pinnae of naive rats. Morphine injected prior to the application of croton oil did not affect the ICS response when assessed by measurements of pinnae thickness. CHS was induced by applying the antigen 2,4-dinitro-1-fluorobenzene (DNFB) to the pinnae of rats which had been sensitized to DNFB. The rats received an injection of mor-

phine prior to either initial antigen exposure (sensitization) or antigen reexposure (challenge). Morphine prior to challenge, but not sensitization, resulted in a pronounced enhancement of the CHS response as measured by pinnae thickness. Quantitative PCR also showed increased levels of IFN-gamma mRNA in the inflamed tissue of morphine-treated rats. Naltrexone blocked the morphine-induced enhancement of the CHS response. The differential effects of morphine suggest that opioids have a more pronounced effect on *in vivo* immune responses which involve immunological memory.

The opposite anti-oedema effects were obtained with NGF, thereby inhibiting the contact hypersensitivity reaction at the elicitation phase.

Example 6. Antipsoriatic, anti-allergic, anti-pruritic, and analgesic effects of NGF.

Nerve growth factor induced the formation of a granular layer in the mouse tail test used as a model of psoriasis. NGF at a concentration of 10-250 µg/ml also exhibited anti-immune activities in the cotton-pellet granuloma assay in rats, in oedema induced by croton oil in mice, and in the peritoneal capillary permeability test in mice.

NGF showed dual effects on analgesia. Both pro (>1000 µg/ml) and anti (<500 µg/ml) analgesic effects were obtained in the model with writhings induced by acetic acid, whereas 50 µg NGF per ml was effective in the hot plate test in mice.

These findings clearly demonstrate the anti-inflammatory, analgesic, and antipsoriatic properties of nerve growth factor.

Example 7. NGF induced blocking of experimental allergic conjunctivitis induced by ICAM-1 (CD54) and LFA-1 (CD11a).

Cell adhesion molecules are critical for the homing and migration of leukocytes into inflamed tissues. The role

of ICAM-1 and LFA-1 was investigated in a previously described experimental model of allergic conjunctivitis induced by ragweed (Rw). SWR/J mice were treated intraperitoneally 6 and 1 h prior to a topical challenge with Rw with injections of an anti-ICAM-1 monoclonal antibody (mAb), an anti-LFA-1 mAb, both anti-ICAM-1 and anti-LFA-mAbs, NGF, or rat IgG. The blocking of ICAM-1 or LFA-1 reduced the clinical signs of allergic conjunctivitis. Treatment with anti-ICAM-1 or anti-LFA-1 mAbs significantly inhibited cellular infiltration into the conjunctiva. However, the greatest inhibitory effect was achieved with NGF at a concentration of 125 μg/ml.

Since NGF significantly inhibits the development of the clinical and histological signs of allergic conjunctivitis, it may be useful for treating patients with ocular allergy.

Example 8. Inhibitory effects of topical administration of NGF on the development of experimental allergic blepharoconjunctivitis in Lewis rats.

FK506 has been used for treatment of cell-mediated immune disorders, such as graft rejection in transplantation or Bechet's disease. In order to evaluate the effectiveness of NGF in another ocular disease model FK506 was injected in rats with experimental allergic blepharoconjunctivitis (EAC), the induction mechanism of which depends on cell-mediated immunity.

Lewis rats were immunized with ovalbumin (OVA) in an emulsion of complete Freund's adjuvant (CFA). NGF was injected intramuscularly daily at 2 μg (n = 8), 20 μg (n = 8) or 200 μg (n = 8) μg from the day of immunization (day 0) to day 6. Control rats were not treated with NGF (n = 8). In addition, 200 μg of NGF was injected from day 7 to day 13 (n = 12) in order to compare the timing of NGF administration (day 0 to day 6, n = 12; control, n = 12). Twenty-

one days after the immunization all the rats were challenged with OVA by eye drops, and 24 h later they were killed after clinical evaluation. Their eyes, blood and draining lymph nodes were harvested for histology, antibody titers and proliferation assay or flow cytometric analysis.

In another set of experiments rats - which had received OVA-primed lymph node cells - did (n = 9) or did not (n = 9) receive additional NGF by daily injections for 4 days. Four days after the transfer these rats were challenged with OVA and evaluated as mentioned above. In order to investigate any possible suppression of disease by the topical administration of NGF both actively immunized and passively immunized rats received OVA together with 0.3% (weight/volume) of NGF (n = 16) or of vehicle (n = 10) by eye drops, and 24 h after challenge the rats were evaluated as mentioned above.

The development of disease, induced by either active or passive immunization, was inhibited in the group which had been treated with NGF, regardless of the timing of the administration. Cellular proliferative responses to OVA were inhibited only in this group. Flow cytometry demonstrated a decrease of about 20% of the total number of CD4-positive T cells. The topical administration of NGF inhibited the development of EAC, though not significantly.

Accordingly, the systemic treatment with NGF, either in the induction or in the effector phase, inhibits the development of EAC in Lewis rats. A topical administration is as effective as a systemic administration.

Example 9. Analgeric effect of NGF on severe corneal melting.

The efficacy of topical treatment with murine NGF was evaluated in 4 patients (Table 1) with severe corneal melting as a consequence of allergic corneal peripheral ulcers. The patients received one drop (about 50 µl) a of NGF solution (10 mg of NGF dissolved in 50 ml of saline so-

lution - 0.9% NaCl) in the conjunctival fornix every two hours (from 6 am to 12 p.m.) for two days, six times a day until the ulcer healed. After the ulcer was completely healed, the dose was reduced to 5 mg in 50 ml of solution four times daily for two weeks. In each case the corneal ulcer completely healed with nerve growth factor treatment.

Case 1

A 56 year old male affected by rheumatoid arthritis with recurrent acute episodes of keratoconjunctivitis and blepharitis which were responsive to topical steroids, developed a peripheral corneal ulcer and scleritis in the left eye. Treatment with systemic steroids and methotrexate failed while a therapy with systemic and topical steroids induced an improvement in eye inflammation and a deterioration of corneal ulcer. The ulcer increased in depth, became enlarged and developed melting and neovascularization. After 5 days of NGF treatment the ulcer displayed a healing process characterized by epithelium growth through the edges of the ulcer. During the first few days of treatment the patient experienced a local increase in pain and photophobia, which progressively disappeared during the healing process together with the inflammatory reaction. The ulcer healed completely after 21 days of treatment.

Case 2

A 63 year old woman underwent cataract extraction and after two months developed necrotizing scleritis with subsequent exposition of choroid. The patient was placed under treatment with topical and systemic steroids and immunosuppressive agents. To avoid the impending risk of eye perforation a scleral patch was performed to cover the area of dehiscence. The patient showed a chronic inflammatory reaction in the postsurgery period despite topical treatment with steroids and systemic immunosuppressive therapy. One month later the patient developed a relapse of the sclero-

malacia which was associated with a peripheral corneal ulcer. As the eye started to develop stromal melting a topical steroid therapy was replaced with non-preserved artificial tears, but the inflammation and the ulcer worsened. Topical application of NGF was begun 5 days later. After 7 days, a conjunctival pannus started to cover the ulcer and a healing process and decrease of the inflammation in the area of scleromalacia became apparent.

During the first week of treatment the patient experienced local pain and photophobia. Two months later, the cornea was completely healed with a pannus in the area of the ulcer and the area of dehiscence was markedly reduced. The eye did not show any signs of inflammation.

Case 3

When treated with ethambutol, rifampicin, and pyrazinamide a 45 year old woman affected by lung tuberculosis developed erythema, small leg ulcers, and bilateral peripheral corneal ulcers associated with scleritis. Conjunctival and skin biopsy confirmed the diagnosis of systemic and ocular vasculitis. Despite a topical and systemic therapy with steroids the corneal ulcers worsened and stromal melting developed. After 7 days the right eye (with the shallower ulcer) was treated with topical 2% cyclosporine 4 times daily, while the left eye (with a pre-Descemet ulcer) was treated with topical NGF. The patient complained of increased pain and photophobia in both eyes.

Two weeks later, the healing process was evident in both eyes, although a marked inflammatory condition was present in the right eye. After 3 weeks of treatment the right eye was completely healed with a conjunctival pannus in the area of the ulcer, although inflammation persisted, while the left eye was completely healed after 4 weeks. A conjunctival pannus developed in the area of the ulcer, and the eye did not show any signs of inflammation. During the following 4 months the patient continued to have a persis-

tent ocular inflammation in the right eye with a corneal punctate keratopathy which was not present in the left eye.

When followed up after 4 months the patient was found to have a relapse of the corneal ulcer in the right eye, which showed a rapid and progressive worsening despite systemic and topical steroids treatment. After 4 days a treatment with topical steroids was replaced with 2% cyclosporine, but no improvement was observed after 10 days of treatment. Treatment with NGF was begun in the left eye, and after 7 days the epithelium begun to grow through the edges of the ulcer. The ulcer healed completely within 2 weeks. A follow-up after 3 months showed no relapse of the ulcer in the right eye.

Case 4

A 62 year old male, affected by recurrent scleritis and responsive to topical steroids developed a peripheral corneal ulcer which was associated with scleritis.

The patient was treated with systemic steroids and acetylsalicylic acid for non-specific arthritis. Topical steroids and systemic immunosuppressive drugs associated with the anti-inflammatory systemic therapy reduced the inflammation but worsened the corneal ulcer, which became deeper and wider while undergoing stromal melting and neovascularization. After five days a topical treatment with NGF was started. During the first days of treatment the patient experienced transient local pain and photophobia.

Healing started after 7 days of NGF treatment, and thereafter the symptoms progressively disappeared. The inflammatory reaction decreased and the healing process was completed after 24 days of treatment.

In conclusion, all corneal ulcers treated with nerve growth factor healed within 8 weeks and no relapse of the disease was observed in any patient during the follow-up (3-12 months) (Table 2). A transient local pain and photophobia was observed during NGF treatment, which preceded

the healing process and disappeared soon after the completion of the healing.

Example 10. Effects of NGF on laminitis.

Laminitis is a major cause of lameness in dairy cattle, goats and horses, and is widely attributed to a defect in the horny tissue that gives the hoof its mechanical strength. Defective horn is associated with, and may be preceded by, impaired keratin deposition in the hoof epidermis.

The hormonal regulation of keratin synthesis and cell proliferation in the bovine hoof was studied using tissue explants in organ culture. As the highest incidence of laminitis is in early lactation, the study focused on insulin, cortisol and prolactin, three hormones implicated in lactogenesis and galactopoiesis. An incubation of tissue explants for 24 h in medium containing insulin (10-5000 ng/ml) stimulated protein synthesis as measured by the incorporation of 35S-labelled amino acids. Histochemical examination showed that the insulin binding co-localized with the site of protein synthesis. Insulin also stimulated DNA synthesis, an index of cell proliferation, which was measured by the incorporation of [3H]methyl thymidine. Cortisol (10-5000 ng/ml) decreased the protein synthesis, whereas prolactin (10-5000 ng/ml) had no significant effect on neither the protein nor the DNA synthesis.

Epidermal growth factor (10-200 ng/ml), a potent inhibitor of keratinization in other tissues, stimulated protein synthesis compared with untreated controls. The binding of epidermal growth factor was located microscopically to the germinal and differentiating epidermal layers.

SDS-PAGE and fluorography showed that the population of proteins synthesized in the presence of any hormone or growth factor combination did not differ from that in untreated controls and included the keratins involved in horn deposition. The results show that bovine hoof keratiniza-

tion is under endocrine and epidermal growth factor control, and suggest that systemic changes in lactogenic hormones may act to inhibit keratin deposition.

Ten lame adult goats with foot lesions were treated with 1000 μg NGF, which was injected locally in the affected foot. All the goats were treated for 5 consecutive days. After the treatment eight were free of symptoms within one week after the end of the treatment.

Seven lame adult cows with foot lesions were treated with 1000 microgram NGF injected locally in the affected foot. All cows were treated for 5 consecutive days. After treatment 6 were free of symptoms within one wek after end of treatment. These cows also had increased milk production.

Table 1 – Characteristics of patients enrolled in the study

Patient No	Age, Sex	Ocular and Systemic history	Ocular findings	Systemic Treatment	Microbiological test	Histopathology	Positive blood exams	Diagnosis
1	56, M	Recurrent keratoconjunctivitis.	Blepharitis, peripheral corneal ulcer in LE	Predinisone (40mg/dag) Methotrexate (7.5mg/wk)	Sterile	-	Rheumatoid factor, ANA, anti-MBO	Rheumatoid arthritis
2	63, F	Cataract surgery 2 months before in LE No-specific arthiritis	Necrotizing scleritis with peripheral corneal ulcer in LE	Prednisone (40mg/dag) Cyclophosphamide (100mg/day)	Sterile	Neutrophils and lymphocytes (corneal cytology)	None	Immune reaction following cataract surgery
3	45, F	Tuberculosis	Scleritis, peripheral corneal ulcers in both eyes	Pyrazinamide (1.25g/day) Ethambutol (1.25g/day) Rifampin (600mg/day) Prednisone (40mg/day)	Sterile	Vasculitis (conjunctival biopsy)	Circulating immunocomplex	Necrotizing vasculitis
4	62, M	Recurrent scleritis	Scleritis, peripheral corneal ulcer in RE	Prednisone (40mg/day) Cyclophosphamide (100mg/day) Acetylsalicylic acid (3.5g/day)	Sterile	-	None	Immune ulcer of unknown cause

LE = left eye; RE = right eye

Table 2 – Effects of NGF treatment

Patient no	Associated systemic treatment	Onset of healing (days)	Complete healing (days)	Side effects (pain and photophobia) days	Follow-up (months)
1	Prednisone (40mg/day) Methotrexate (7.5mg/wk)	5	21	6	7
2	Prednisone (40mg/day) Cyclophosphamide (100mg/day)	7	60	9	12
3	Pyrazinamide (1.25g/day) Ethambutol (1.25g/day) Rifampin (600mg/day) Prednisone (40mg/day)	14	28	9	8
4	Prednisone (40mg/day) Cyclophosphamide (100mg/day) Acetylsalicylic acid (3.5g/day)	7	24	8	4

CLAIMS

1. Use of nerve growth factor for the manufacturing of a drug for treating allergic disorders in human beings and animals.

2. Use as in claim 1 by administration of the drug to the site of action at a nerve growth factor concentration of up to 1,000 µg/ml.

3. Use as in claim 1 or 2 by administration of the drug to the site of action at a nerve growth factor concentration which does not result in the perception of pain.

4. Use as in claim 3, characterized in that the nerve growth factor concentration is less than 100 µg/ml.

5. Use as in any of claims 1 to 4, characterized in that the allergic disorder is a local or systemic disease.

6. Use as in claim 5, characterized in that the local or disease is a corneal ulcer.

7. Use as in any of claims 1 to 4, characterized in that the allergic disorder is selected from the group comprising allergic inflammatory arthritis, osteoarthrosis, osteoporosis, defect fracture healing, venous ulcer, diabetic ulcer, decubitus pressure ulcer, conjunctivitis, dermatitis, and hypersensitivity to drugs.

8. Use as in claim 7, characterized in that the allergic inflammatory arthritis is selected from the group comprising rheumatoid arthritis, juvenile arthritis, systemic lupus erythematosus, systemic sclerosis, polymyositis, necrotitizing vasculitis, Chron's disease, ulcerous colitis, irritable bowel syndrome, degenerative changes in the gastro-intestinal tract, degenerative bladder, degenerative changes in the bladder, Sjogren's disease, psoriasis, and sarcoidosis.

9. Use as in any of claims 1 to 4, characterized in that the allergic disorder is a contact

allergy of the type conjunctivitis, dermatitis, and hypersensitivity to drugs.

10. Use as in any of claims 1 to 4, c h a r a c - t e r i z e d in that the allergic disorder is a allergic lesion in the skin.

11. Use as in any of claims 1 to 10, c h a r a c - t e r i z e d in that the drug is systemically supplied to the site of action.

12. Use as in any of claims 1 to 10, c h a r a c - t e r i z e d in that the drug is locally supplied to the site of action.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/02698

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 38/18, A61P 37/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Int Arch Allergy Immunol, Volume 118, 1999, S. Bonini et al, "Nerve Growth Factor: An Important Molecule in Allergic Inflammation and Tissue Remodelling", page 159 - page 162, page 161, right column, lines 7-15 --	1-12
X	Microsc. Res. Tech., Volume 45, 1999, L. Aloe et al, "Nerve Growth Factor: A Neurotrophin With Activity on Cells of the Immune System", page 285 - page 291, page 287, left column --	1-12
X	WO 9943839 A1 (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA), 2 Sept 1999 (02.09.99), page 7, line 22; page 23, lines 3-15 --	1-12

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"P" document published prior to the international filing date but later than the priority date claimed
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 May 2001	06 -06- 2001
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/02698

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	STN International, File CAPLUS, CAPLUS accession no. 1998:254251, Document no. 129:53112, Nagai, Hiroichi et al: "Nerve growth factor and allergies"; Arerugi no Ryoiki (1998), 5(3), 318-324	1-12
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A	Dialog Information Services, File73, EMBASE, Dialog accession no. 06526222, Embase accession no. 1996191584, Berczi I. et al: "The immune effects of neuropeptides"; & Bailliere's Clinical Rheumatology (BAILLIERE'S CLIN. RHEUMATOL.) (United Kingdom) 1996, 10/2 (227-257)	1-12
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A	WO 9846254 A1 (BOEHRINGER MANNHEIM GMBH), 22 October 1998 (22.10.98), page 2, line 4 - line 10; page 5, line 27 - line 33, claims 14-15	1-12
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INTERNATIONAL SEARCH REPORT

Information on patent family members

30/04/01

International application No.
PCT/SE 00/02698

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		BR 9908267 A	24/10/00
		EP 1078093 A	28/02/01
WO 9846254 A1	22/10/98	AU 7214998 A	11/11/98